

## **Cattle Molecular Markers and Parentage Testing Workshop**

**Chair:** Leanne van de Goor, VHL, The Netherlands

**Approximate number of attendees:** 75

**Report on any business conducted:** The Cattle Molecular Markers and Parentage Testing (CMMPT) workshop was held on July 28<sup>th</sup> 2010, and focused on discussion of DNA typing results for the 2009-2010 Cattle Comparison Test (CT), implementing a rating system for the results of CT, the development of a SNP panel suitable for parentage verification, Water Buffalo CT and Llama/Alpaca CT.

### **2009-2010 Cattle CT**

The Duty lab work for the CT was done by Tara McParland/Aron Weir (Maxxam, Canada). DNA was extracted from twenty blood/semen samples (originating from Holstein, Jersey and Ayrshire). A reference DNA sample with genotypes for 42 STR markers was provided. The samples were not selected with variation in the genotypes for Non-microsatellite markers (e.g. CVM and BLAD). The extracted DNA was sent to 74 labs. Four labs requested and received new samples. Issues with blocked/missing consignment forms sent by email and/or fax made application difficult. Also the duty lab received several consignment forms that were incomplete or incorrectly filled out (courier or membership data absent or not updated, several import permits were sent late). Plans by the ISAG's Executive Committee (EC) to implement online application for CTs via the society's website should alleviate these problems for future tests. Under the new system, labs would be able to log in via their active institutional membership ID and only completely filled out consignment forms will be accepted. It was suggested to ISAG's EC to make this service available for the next CT. The computer work was performed by Dr. Luis Cancela (Identitas, Uruguay). Results were received from 74 labs representing 32 countries. Fourteen of these were labs participating for the first time in the Cattle Comparison Test. Since several lab ID codes are circulating, lab identification was difficult. For the next CT only the new 5 digit lab ID from FASS will be accepted. The 12 STRs in the ISAG-recommended panel (BM1818, BM1824, BM2113, ETH3, ETH10, ETH225, INRA23, SPS115, TGLA53, TGLA122, TGLA126 and TGLA227) were reported by 59 labs. Among the remaining markers, 6 markers were reported by far more labs than the other STR markers: MGTG4B (24), CSRM60 (24), SPS113 (22), ILSTS006 (20), RM67 (19) and CSSM66 (18). As in previous comparison tests, there was low interest in non-microsatellites markers (diagnostic markers), e.g. CVM and BLAD. Thirteen diagnostic markers were reported, ranging from 1 to 8 labs per marker.

The absolute (Aga) and the relative (Rga) genotyping accuracies were calculated according to the ISAG suggested guidelines

([http://www.isag.org.uk/Docs/2009\\_IFAG\\_ISAG\\_CTRatingSystem.pdf](http://www.isag.org.uk/Docs/2009_IFAG_ISAG_CTRatingSystem.pdf)).

The overall concordance among labs for the 12 ISAG-recommended markers was good. Because 15 labs did not report results for all the twelve ISAG markers the percentage of labs with Rank 1 for Rga was higher than the percentage of labs with Rank 1 for Aga, as shown in table below.

Rank	Aga (% Labs)	Rga (% Labs)
1: 100 – 98%	58	72
2: 98 – 95%	12	15
3: 95 – 90%	11	7
4: 90 – 80%	16	4
5: <80%	3	2

There was very good agreement in the parentage verification questions, with 70 labs reporting the correct answers.

For the 2011-2012 CT, John Flynn/Romy Morrin-O'Donnell (Weatherbys, Ireland) volunteered to serve as Duty Lab. Dr. Luis Cancela (Uruguay) volunteered to serve again as the Computer Lab. There was not enough interest among the participants of this workshop (only three participants indicated their interest by show of hands) to organize a separate CT test for non-microsatellite markers in 2011-2012.

### **Implementation rating system for the results of CT**

It was agreed by the workshop to implement the suggested rating system for the results of the CT already for the 2009-2010 CT. It was agreed by the workshop that for the 2011-2012 CT only the labs that report at least the 12 ISAG-recommended markers will be included in the accuracy calculations. On the consignment form, participants should be able to choose whether their results will be included in the accuracy calculations.

### **Development of a SNP panel suitable for parentage verification**

Dr. Cecilia Penedo (UC Davis, USA) gave an update about the evaluation of SNP testing methods and testing platforms for animal identification and parentage verification. During a meeting in Amsterdam (June 23-24, 2009), 11 participants discussed the application of the SNP technology in cattle. In order to evaluate platforms and test performances, a CT for SNPs was organized. It was agreed that samples would be tested in triplicate by each lab and that different platforms would be used. Forty DNA samples were distributed, and six laboratories reported results. Eight different platforms were used (Kbioscience Caspar, Sequenom I-Plex, Sequenom Platinum, Illumina, Fluidigm, Taqman Open Array, Illumina Golden Gate, Illumina Multi-Sample-Indexing). The results are currently being compiled and the report will be available by the end of 2010. After this workshop, the comity had a meeting jointly to ICAR, in witch it was decided to organize a SNP comparison test in 2011.

### **2010 Water Buffalo CT**

Dr. Daniela Iamartino (Italy) gave a presentation about the first STR CT in Water Buffalo started in March 2010. The Duty lab was AIA-Laboratorio Genetica e Servizi (Cremona, Italy) and the Computer lab was Parco Tecnologico Padano (Lodi, Italy). DNA was extracted from twenty blood samples of River Buffalo (*Bubalus bubalis*, 2n=50) originating from Mediterranean Italiana and Murrah breeds. The DNA sample BUF-CT1 was used as reference with genotypes for 20 STR markers provided. Twenty DNA samples were shipped to 8 labs and results were returned by 4 labs. The Duty lab received some consignment forms that were incomplete (without courier name, without international account number, etc.) and several incomplete import permits. The samples returned from one lab. The new plans of ISAG's EC for online application should resolve these problems in the future. The two STR panels, the primary one of 14 markers (MAF65, INRA006, CSSM047, CSSM019, RM4, CSSM042, CYP21, BMC1013, BMS922, INRA026, CSSM060, CSSM038, BM1706, CSSME70) and the secondary one of 6 markers (TGLA227, ETH003, BM1824,

FCB304, BM757, CSSM033), were suggested based on observed allele frequencies in 30,000 river buffaloes and they are cattle and sheep derived microsatellites. One lab sent results for all suggested markers, one for 16, one for 15 and one for 13 for a total of 10 in common markers (BM1706, BMC1013, CSSM019, CSSM038, CSSM042, CYP21, INRA006, INRA026, MAF65 and RM4). It was observed any marker failure, no *large scale* problems with amplification except for RM4 marker with some missed alleles. There were many discrepancies in 2-4 base pair reading shift for BMC1013, CSSM047, INRA006, RM4 and TGLA227. The concordance among labs for the analyzed markers was good. The marker performance was good for all markers except for RM4. There was very good agreement in the offspring verification questions with all labs reporting the correct answers.

For the next 2011-2012 CT, during the Workshop Dr. Elena Genzini (AIA-LGS, Italy) volunteered to serve as Duty lab and Dr. Daniela Iamartino (PTP, Italy) volunteered again to serve as Computer lab following the ISAG suggested guidelines.

### **2008-2009 Llama/Alpaca CT**

A meeting of the Llama/Alpaca Genotyping Working Group was held at the end of the cattle workshop. Dr Cecilia Penedo (UC Davis, USA) gave a presentation about the first STR CT in Llama/Alpaca, held in 2008-2009. The Duty and Computer lab was the Veterinary Genetics Laboratory (UC Davis, USA). Forty DNA samples (22 alpacas and 18 llamas) were shipped to 18 laboratories. Results were returned by 15 laboratories. A separate report will be filed for the Llama/Alpaca CT.

### **Election of standing committee**

Elena Genzini completed her term at the Standing Committee. Daniela Iamartino was elected during the workshop to fill the vacant position. The new committee is composed of: Leanne van de Goor (Chair), Cecilia Penedo, Romy Morrin-O'Donnell, Marcela Martinez, Marie-Yvonne Boscher and Daniela Iamartino. John Flynn/Romy Morrin-O'Donnell join the committee as Duty lab and Luis Cancela as Computer Lab.